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CHEMICAL EXAMINATION

OF

CASCARA BARK

BY

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6. The assay of preparations containing Pilocarpine
7. Pilocarpine and the alkaloids of Jaborandi Leaves
8. A new glucoside from Willow Bark
9. The constitution of Pilocarpine Part I.
10. The composition and determination of Cerium Oxalate
11. Researches on Morphine—Part I.
12. Observations relating to the Chemistry of the British Pharmacopœia
13. Mercurous Iodide
14. The composition of Berberine Phosphate
15. A contribution to the Pharmacognosy of official Strophanthus seed
16. The chemistry of the Jaborandi Alkaloids
17. A new admixture of commercial Strophanthus Seed
18. Researches on Morphine—Part II.
19. The constitution of Pilocarpine —Part II.
20. The chemistry of the bark of Robinia Pseud-acacia, *Linn.*
21. The anatomy of the bark of Robinia Pseud-acacia, *Linn.*
22. A soluble Manganese Citrate and compounds of Manganese with Iron
23. The chemical characters of so-called Iodo-tannin Compounds
24. The constitution of Pilocarpine —Part III.

(Continued on page 3 of cover)

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CHEMICAL EXAMINATION OF CASCARA BARK.

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Although it is about twenty-six years since cascara sagrada was introduced into medicine, our knowledge of the chemistry of this drug is still in a most confused and unsatisfactory condition, and this confusion is increased rather than diminished by reference to the memoirs on the subject. The object of the present investigation was to endeavor to remove this confusion, so far as possible, by a critical review of the literature on the subject, and by repeating the work of previous investigators, as also by a complete chemical examination of genuine barks of known origin.

The first chemical examination of the bark was made by Prescott,* who isolated from *Rhamnus purshianus* a crystalline substance and three resins, besides certain constituents common to most barks, such as a volatile and fatty oil, oxalic and tannic acids, wax, starch, etc. The crystalline substance was obtained from the lead acetate precipitate by decomposing the latter with hydrogen sulphide, and was stated to crystallize from its alcoholic solution in white, double pyramids. It melted and sublimed at a temperature a little above 100° C., was sparingly soluble in petroleum ether, ether, chloroform, or alcohol, but was soluble in benzene. It was neutral to test paper. It was not analyzed, and its description, as given above, affords no clue to its identity. It has not been observed by subsequent investigators, and working by the method detailed by Prescott, I have been unable to obtain any substance of corresponding characters.

Limousin † considered that the resins obtained by Prescott ‡ were derived from chrysophanic acid, which he believed to be present in notable quantity. The experimental evidence for this statement was the fact that the grated surface of the bark was colored red when moistened with potassium hydrate solution or aqueous ammonia. As chrysophanic acid does not yield a red color with ammonia, Limousin's deduction is obviously incorrect, but the reactions above mentioned are characteristic of emodin, which, as will be shown later, is certainly present in the bark. Wenzell § isolated from the bark a small quantity of an orange-red, crystalline substance, melting at 226–230° C., and having the properties of a glucoside. He considered that it was not identical with either frangulin or emodin. No indication was given in the paper of the purity of this

* Amer. Journ. Pharm., 1879, *51*, 165.

† Journ. de Pharm. et de Chim., 1885 (V), VI, 80.

‡ *Loc. cit.*

§ Pharm. Rundschau, 1886, *4*, 79.

substance, and later investigators have shown that it was impure emodin. Meier and Webber * stated that, after an exhaustive examination of the drug, they found a ferment, glucose, and a trace of ammonia, and that the glucoside may be separated by precipitating an aqueous infusion with lead subacetate. As this paper contains no experimental evidence of the identity of the above constituents, it requires no further comment. It contains, however, the first reference I have been able to find respecting the griping alleged to be caused by the use of immature bark, and it will be convenient to deal with this point in this part of the paper. Meier and Webber refer to papers by Baildon † and by Lamm and Fristedt ‡ as authority for the statement that cascara bark when fresh gives rise to a griping action which is not produced by the mature bark. Reference to these papers shows, however, that they contain opposite and contradictory statements, Baildon stating that the bark was "a gentle aperient without griping," whilst Lamm and Fristedt observed griping effects, but in both cases *Rhamnus frangula* and not *purshianus* was referred to.

Moreover it has been stated § that "to obtain the best results, the bark (*Rhamnus purshianus*) must be of comparatively recent collection." Meier and Webber state that fresh cascara bark contains a ferment which seemed to be identical with that existing in cabbage, licorice root, and other vegetables, and that in the stomach this ferment forms lactic acid, which causes the griping. In opposition to this it is now known that the formation of lactic acid is due, not to the action of a vegetable ferment, but to the lactic acid bacillus (*B. acidi lactici*), and this explanation of the griping action of the drug must therefore be abandoned.

Moss and Jardine ‖ examined the therapeutic effect of barks of known origin, collected at different seasons of the year, from different localities, and of different age, but their results were somewhat contradictory. In the summary Jardine stated: "With the exception of fluid extract of bark collected in the spring of 1890, I could not distinguish much difference in one extract from another." That collected in the spring of 1890 showed a marked griping action, but that of more recent collection, August 1890, did not. It is clear, therefore, that these results do not prove that the griping action is due to the use of immature bark.

Schwabe ¶ examined *Rhamnus purshianus* and found emodin, identical with that obtained from *Rhamnus frangula*, to exist as such in the bark, and identified it by means of its acetyl and dibromo compounds, all of which were analyzed. He considered that Wenzell's crystals, previously referred to, were merely impure emodin, and could obtain no evidence of

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* Amer. Journ. Pharm., 1888, *60*, 87.

† Year Book of Pharmacy, 1871, 560.

‡ Zeit. Oesterr. Apoth. Ver., 1876, 156.

§ Year Book of Pharmacy, 1886, 168.

‖ Year Book of Pharmacy, 1891, pp. 476, 482.

¶ Arch. Pharm., 1888, *226*, 569.

the existence of a glucoside, nor could he isolate any other crystalline substance.

Zeig * further examined the resins previously described by Prescott, but was unable to isolate any definite principle.

La Prince † claimed to have obtained the active principle of cascara bark in a crystalline form. It was stated to have been prepared by extracting the bark by boiling with an aqueous solution of sodium carbonate, neutralizing the strained decoction with acid, filtering, and evaporating the filtrate to dryness in a vacuum. The residue was extracted with acetone, the acetone solution acidulated with sulphuric acid, and then poured into a large excess of water. The yellow, crystalline deposit which separated was purified by a repetition of the above process. This substance, which he named *Cascarine*, formed microscopic, prismatic needles, which darkened at 200° C. and melted at 300° C. with decomposition. It was tasteless, insoluble in water, and dissolved in alkalies with the formation of a purple-red color. It gave on analysis numbers agreeing with the formula $C_{12}H_{10}O_5$, but the analytical details were not given. When fused with potassium hydroxide a small amount of a substance was formed which he considered might be phloroglucinol. Finally Le Prince suggested that cascarine may be identical with rhamnetin.

A most curious confusion has arisen in chemical literature with respect to this substance. Beilstein, under cascarine,‡ queries it as identical with rhamnetin, but Phipson § considered that it was identical with xanthorhamnin, and Van Rijn,‖ without comment, accepts this latter conjecture, and under xanthorhamnin gives the details of Le Prince's preparation of cascarine from cascara.

The properties of cascarine, as given by Le Prince, prove that it could *not* be identical with either rhamnetin or xanthorhamnin, and this is clearly shown in the following table :

Property.	Cascarine.	Rhamnetin.	Xanthorhamnin.	Emodin.
Composition	$C_{12}H_{10}O_5$.	$C_{16}H_{12}O_7$.	$C_{48}H_{66}O_{29}$.	$C_{15}H_{10}O_5$.
Melting-point	300° C.	——	——	256° C.
Solubility in water..	Insoluble.	Insoluble.	Extremely easily soluble.	Insoluble
Color with alkalies..	Purple red.	Yellow.	Yellow.	Purple-red.

* Proc. Amer. Pharm. Assoc., 1889, *37*, 261.

† Compt. rend., 1892, *115*, 286.

‡ Handbuch, 3d ed., iii, 627.

§ Compt. rend., 1892, *115*, 474.

‖ "Die Glykoside," 1900 ed., 299.

Le Prince presents no evidence of the purity of cascarine, and it agrees in properties, with the exception of the melting point, with emodin. Furthermore, by the method of preparation adopted, the resulting product would almost certainly contain emodin. It is, therefore, exceedingly probable that the cascarine of Le Prince was nothing more nor less than impure emodin. Le Prince's experiments have been carefully repeated, but, as will be shown later, it has not been possible to isolate any substance agreeing in properties with cascarine.

Dohme and Engelhardt* examined *Rhamnus purshianus*, and claimed to have isolated the active principle of the drug, which they named *Purshianin*. This was stated to be a glucoside, yielding on hydrolysis emodin, and a sugar which was not identified. Before dealing with this substance there are some other points in this paper to which attention may be directed.

In the first place Schwabe's results are incorrectly recorded by them, since in a pamphlet entitled "The History, Pharmacognosy and Chemistry of Cascara Sagrada," p. 3, they make the following statement: "He (*i. e.*, Schwabe) unquestionably errs as far as buckthorn is concerned, as Lieberman and Thorpe each actually obtained frangulin, a glucoside melting at 225° C., from buckthorn, and from this frangulin, by saponification, emodin."

Schwabe,† as a matter of fact, found both frangulin and emodin in buckthorn bark (*Rhamnus frangula*), and, as evidence of this, the concluding sentence of Thorpe's last paper may be quoted: ‡ "We conclude, therefore, that there can no longer be any doubt that the formula $C_{21}H_{20}O_9$, which was first assigned to frangulin by Schwabe, is correct, and that his equation,

$$C_{21}H_{20}O_9 + H_2O = C_{15}H_{10}O_5 + C_6H_{12}O_5,$$

truly represents its hydrolysis."

Thorpe and Miller also found emodin in frangula bark, in addition to frangulin, thus confirming Schwabe's statements. Dohme and Engelhardt also state: § "The investigations of the author (Dohme) and Dr. Engelhardt of this laboratory, as given in detail below, also show that Schwabe erred as far as cascara is concerned, for they obtained from it not emodin as a first product, but a glucoside melting at 237° C., crystallizing in dark, red-brown needles, which they named purshianin, and which on saponification by treatment with dilute acids yielded emodin and a sugar."

Schwabe's results, already referred to, are quite conclusive with regard to the identification of emodin, and no experimental evidence is offered by Dohme and Engelhardt in support of their assumption that emodin is not present in cascara. Moreover, Schwabe's results have been confirmed

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* Proc. Amer. Pharm. Assoc., 1897, *45*, 193.

† *Loc. cit.*

‡ Thorpe and Miller, *J. C. S.*, 1892, *61*, .

§ Pamphlet, p. 3.

by Le Prince * and by the author, and the experimental details, given in another part of this paper, show that emodin can be extracted directly from cascara bark by benzene, thus avoiding all possibility of hydrolysis. In fact, part of the emodin used by the author in another investigation † was prepared in this way directly from cascara bark.

Dohme and Engelhardt examined the fatty oil of cascara, and considered it to consist of a mixture of dodecyl palmitate and stearate. ‡ The evidence of the identity of dodecyl alcohol seems fairly conclusive, although the purity of the substance melting at 24°–26° C., and subsequently analyzed was not assured. On the other hand, the evidence of the identity of the fatty acids with palmitic and stearic acids is very inconclusive, and the authors themselves admit that this point cannot be considered definitely settled.

The most important part of Dohme and Englehardt's paper is, however, their statement that cascara does not contain emodin, but a glucoside, purshianin, yielding emodin on hydrolysis, and that this is the active principle of the drug.

I shall proceed to deal somewhat in detail with the evidence adduced for the existence of this glucoside. It was prepared by extracting the drug, first with chloroform, to remove fat, etc., and then with 80 per cent. alcohol. After distilling off the alcohol, the residual extract was dissolved in water and precipitated with lead subacetate. The latter precipitate was then decomposed by hydrogen sulphide, and the filtrate from the lead sulphide evaporated. In this way a hard brown-red substance was obtained, which, they state, "is very difficult to obtain in a crystalline form, as efforts to crystallize it from acetone and ethyl acetate resulted only in our obtaining a few dark brown-red needles, melting at 237° C., the most of it separating in an amorphous condition. Not sufficient of it was obtained to make an analysis, but we could confirm that it was not emodin, as it gave no purple color on being treated with caustic potash. It is the glucoside of cascara sagrada."

It will be observed that no proof was given of the purity of the crystals (m. p. 237° C.), and the substance used for hydrolysis was apparently the amorphous product.

The entirely unwarranted assumption was made that this amorphous substance was homogeneous and identical with the crystals melting at 237° C. If this had been the case, the greater part of it would undoubtedly have crystallized. From a consideration of the work of previous investigators, it would appear likely that these crystals were impure emodin, but it is stated that they were not emodin because they gave no purple color with caustic potash. The actual color reaction obtained is not stated, but

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* Compt. rend., 1899, *129*, 60.

† Jowett and Potter, *J. C. S.*, 1903, *83*, 1327.

‡ *Loc. cit.*, p. 197.

it would be expected that the impurity present might modify this color reaction to some extent. Indeed, a few lines further on they state that emodin gives a blood-red color with caustic alkali. There is, therefore, no evidence whatever adduced to show that these crystals were not impure emodin, nor is any proof furnished of the glucosidal character of this substance. No tests for the presence of sugar previous to the hydrolysis are recorded, and it is difficult to see on what basis the assumption rests that the sugar afterwards found was produced by the hydrolysis of the amorphous substance. Finally, with regard to the emodin isolated, there is no proof that it was not present as such in the original substance, as only a portion may have crystallized out owing to the presence of other substances which prevented crystallization. By treatment with acid these associated substances might have undergone change, so that the emodin was then more easily isolated. Experimental evidence of the correctness of this explanation will be found in a later portion of this paper. It is clear that no experimental evidence has been adduced to prove the existence of purshianin.

Dohme and Engelhardt also attempted to obtain the bitter principle in a crystalline form, but were unsuccessful.

Le Prince* stated that he had isolated from the bark, emodin, chrysophanic acid, and chrysarobin. The evidence which he adduced of the presence of the two latter substances is by no means conclusive. The chrysarobin was stated to have been extracted from a crude product by a small quantity of acetic acid, and its melting-point was given as 165°–170° C., but it was not analyzed. If this substance were chrysarobin it was evidently impure, as pure chrysarobin† is sparingly soluble in acetic acid, and melts at 204° C.

The chrysophanic acid was separated by Le Prince by using a larger volume of acetic acid. It was found to melt at 160°–162° C., gave the theoretically required figures on analysis, and dissolved completely in ammonia with a red coloration. Chrysophanic acid (*loc. cit.*), however, is known to melt at 190° C., and is insoluble in ammonia. Whilst these results indicate that substances allied to emodin were possibly present, their identity with chrysophanic acid and chrysarobin cannot be considered definitely established.

Aweng‡ has stated that the bark contains primary and secondary glucosides, yielding on hydrolysis, emodin, chrysophanic acid, and rhamnetin. As, however, he does not regard these glucosides as definite substances, and furnishes no experimental evidence to prove either their glucosidal nature or the identity of the products of hydrolysis, his results need not here be further considered.

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* Compt. rend., 1899, *129*, 60.

† Jowett and Potter, *J. C. S.*, 1902, *81*, 1577.

‡ Pharm. Centralhalle, 1898, 776; Apothek. Zeit., 1900, *15*, 537; 1902, *17*, 372.

Cascara contains a very small amount of a volatile oil, which has been examined,* but the amount present is so small that it would not appear to be of much importance as a constituent of the bark.

In view of the conflicting statements which have just been referred to, it may be advantageous to state concisely the results of the critical examination of the literature on this subject.

1. The only definite principle isolated from cascara bark, the identity of which can be considered to be absolutely established, is emodin.

2. The statement of the existence in the bark of chrysophanic acid, chrysarobin, or glucosides yielding on hydrolysis emodin, chrysophanic acid or rhamnetin, is not supported by satisfactory experimental evidence.

3. Wenzell's "crystals," Le Prince's "cascarine," and Dohme and Engelhardt's "purshianin" would appear from the description given by the respective authors to be merely impure emodin.

4. No indication can be given of the identity of the crystals described by Prescott.

5. It has been stated by Dohme and Engelhardt that the fat of cascara consists of dodecyl palmitate and stearate.

In the course of the present detailed examination of the constituents of the bark, especial attention has been directed to the above points. Particular care has been taken to ensure the authenticity of the barks examined, and, where definite substances have been isolated, their purity has been established, wherever possible, by the usual chemical methods.

As the details of the experimental work are somewhat extended and are given in the second part of the paper, a summary of the results obtained may appropriately be inserted here, the following numbers corresponding with those employed in the above summary of previous work on the subject:

1. In addition to emodin, the presence of which in the bark was fully confirmed, there was isolated a small amount of a substance isomeric with emodin, melting at 183° C., but insoluble in ammonia. It may be identical with the iso-emodin obtained by Thorpe and Miller from frangula bark.† Its acetyl derivative melted at 168° C.

Glucose was also proved to occur in the bark, and a substance which, on treatment with acid, yielded syringic acid. It has been proved that the latter does not exist as such in the bark, but is formed by the action of acids. The nature of the compound yielding it could not be ascertained.

2. No evidence whatever could be obtained of the existence of chrysophanic acid or chrysarobin in the bark, or of glucosides yielding on hydrolysis emodin, chrysophanic acid or rhamnetin. A possible explanation of the results of previous observers would seem to be afforded by the peculiar behavior of emodin. It was found, for example, that emodin,

* Haensel, *Apoth. Zeit.*, 1901, *16*, 754.

† J. C. S. 1892, *61*, 6.

although insoluble in water, is soluble in the aqueous extract of the bark, and that it is extracted from such a solution only slowly and with difficulty by shaking with immiscible solvents such as benzene, ether or chloroform. On the other hand after treatment with acids, the water-soluble substances were decomposed with formation of insoluble resins, and the emodin was very readily extracted from such a mixture. This curious behavior, recalling that of the digitalis glucosides, might lead to the deduction that glucosides yielding emodin on hydrolysis were present. If, however, the aqueous extract is repeatedly shaken with chloroform or ether, to remove the greater part of the emodin, and then hydrolyzed, not more than traces of emodin will be found.

Schwabe's conclusions as to the presence of emodin and the absence of a glucoside yielding emodin on hydrolysis were thus completely confirmed.

3. On repeating the experiments of Le Prince and of Dohme and Englehardt it was not possible to isolate a pure substance corresponding to either cascarine or purshianin. There is no doubt but that these represent impure products and not chemical entities.

4. The efforts to obtain the crystals described by Prescott were unsuccessful.

5. The bark contained about two per cent. of a fat which consisted of rhamnol arachidate, free arachidic acid, and substances, probably glycerides, yielding on hydrolysis linolic and myristic acids. The name *rhamnol* has been assigned to the alcohol, $C_{20}H_{34}O$ melting at 135° to 136° C., which is combined with arachidic acid in cascara, and is identical with an alcohol obtained from Kô-sam seeds by Power and Lees.* Its acetyl derivative melts at 117° C. Rhamnol belongs to the type of alcohols of which quebrachol (with which it is possibly identical) cupreol and cinchol are members.†

6. Attempts to obtain the bitter principle or derivatives of it in crystalline form were unsuccessful.

7. No difference could be observed between the chemical characters of the fresh (one year old) or of the so-called matured bark (3 years old).

8. Beyond slight differences in the amounts of extractive, etc., the examination of *Rhamnus purshianus* and of *Rhamnus californicus* gave identical results.

9. A hydrolytic enzyme was isolated which hydrolyzed amygdalin, but when administered in 1 gramme doses it had no griping action.

10. The physiological experiments made for the purpose of locating the active principle of the drug resulted as follows:

Emodin is *not* the active principle of the drug, and exerts very little influence, if any, on the characteristic aperient action of cascara.

The active principle or principles producing the aperient action of the

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* Year Book of Pharmacy, 1903, p. 503.

† Beil. Handbuch, 3rd Edit., II, 1068.

drug are contained in that portion of the lead subacetate precipitate extracted by ethyl acetate (see page 17), and which is soluble in water and in alcohol.

No crystalline product could be isolated from this extract, and therefore no clue whatever could be obtained as to the chemical nature of the active principle.

EXPERIMENTAL.

The material used in this investigation, with the exception of specimen 2, was specially collected for the purpose under the supervision of a competent botanist. It consisted of:

1. *Rhamnus purshianus.* Bark collected in Oregon, on 10th September, 1902, and its chemical examination commenced early in 1903.

2. *Rhamnus purshianus.* A carefully selected commercial specimen which was known to be at least three years old.

3. *Rhamnus californicus.* Bark collected in California, in April and May, 1902.

EXAMINATION OF SPECIMEN 1.

Preliminary Experiments.

The powdered bark, after drying at 100°, gave on ignition, 4.3 per cent. of ash, and, after boiling four times successively with water, 32.5 per cent. of dried aqueous extractive.

A determination of tannin by the usual method showed that 2.4 per cent. was absorbed by hide powder.

When extracted successively with the under-mentioned solvents in a Soxhlet apparatus, the bark yielded the following amounts of extract, dried at 100° C. until of constant weight:

(1) Petroleum (b. p. 40–50° C.) = 2.0 per cent.
(2) Benzene = 1.2 per cent.
(3) Ethyl acetate = 23.9 per cent.
(4) Alcohol = 6.3 per cent.

The petroleum extract, which was a brownish-yellow, soft fat, was boiled with a small quantity of alcohol and filtered; the filtrate, on standing, deposited no crystals. The residual fatty matter, left after extraction with alcohol, was digested with a five per cent. cold aqueous solution of potassium hydroxide, and filtered. The filtrate, which was only slightly colored, was acidified, and the acid liquid extracted with petroleum. On removing the petroleum, no appreciable residue remained. There was, therefore, no appreciable amount of chrysarobin or chrysophanic acid present.

The benzene extract was of a dark-red color, and when dissolved in glacial acetic acid yielded emodin (m. p. 250° C.).

The ethyl acetate and alcoholic extracts also had a dark-red color, and showed no tendency to crystallize.

Isolation of a hydrolytic enzyme. The bark was cut into small pieces, digested with cold water for two days, and then strained. The dark-brown

filtrate became turbid on boiling, and gave a slight reaction with the usual alkaloidal reagents. To this aqueous liquid twice its volume of alcohol was added, and the precipitate collected and dried. The yield varied a little in different experiments, but averaged about 1 per cent. of the bark taken. The product was soluble in water, but gave no reaction with the usual alkaloidal reagents.

When a small portion was added to a solution of amygdalin, and allowed to stand at the ordinary temperature for a few hours, the odor of benzaldehyde was developed, thus proving the presence of a hydrolytic enzyme.

Separation of the Constituents of the Bark.

In order to effect a preliminary separation of the constituents of the bark, the following procedure was adopted. The powdered bark was thoroughly extracted with hot alcohol in a reflux apparatus and the alcohol removed by distillation. The resulting dried extract was a dark-brown hygroscopic mass, and amounted to 33.3 per cent. of the bark taken. About five kilos of the extract were mixed with a large volume of water, when a quantity of a dark-brown fatty substance separated (subsequently referred to as A). This was filtered off with some difficulty, and the filtrate extracted by shaking many times with chloroform. It was evident that the last traces of emodin or allied substance were removed very slowly, as the chloroform extract, even after 40 extractions, still gave a very slight coloration to the aqueous layer when shaken with ammonia. The liquid, after extraction with chloroform, was first precipitated with lead acetate solution, when a dirty-colored precipitate was thrown down; this was separated, and the filtrate precipitated with lead subacetate solution, when a very voluminous brick-red precipitate was produced, which was filtered off, and the filtrate freed from lead by hydrogen sulphide.

The fatty substance (A) was next thoroughly extracted with boiling petroleum, when, after distillation, a brown, fatty mass was obtained. The portion remaining undissolved by the petroleum was dissolved in alcohol, the alcohol distilled off, and the concentrated extract poured into a large volume of water. The resin which separated was collected, washed, and dried.

By this treatment the original alcoholic extract was resolved into the following portions, which were further examined:

1. Petroleum extract, chiefly fat.
2. Resin.
3. Chloroformic extract.
4. Lead acetate precipitate.
5. Lead subacetate precipitate.
6. Filtrate from lead subacetate precipitate.

The residual marc, left after extraction with alcohol, was boiled with water, when a dark-brown extract was obtained, which, when evaporated, formed an almost black, amorphous residue. The aqueous solution was tasteless, did not reduce Fehling's solution, and was not further examined.

1. *Examination of the Petroleum Extract.*

The dark-brown, fatty mass was dissolved in alcohol, and hydrolyzed with alcoholic potassium hydroxide, on the addition of which it formed a red solution. After hydrolysis the alcohol was distilled off, and the residue mixed with sand, dried, and extracted in a Soxhlet apparatus with ether.

Neutral Constituents of the Fat.

The ethereal solution, on distillation, left a residue which quickly became crystalline, and after one crystallization from alcohol melted at 131° C. It was then recrystallized, first from alcohol, and subsequently from glacial acetic acid, until of constant melting-point. It was thus obtained in white needles, melting at 135°–136° C. It was sparingly soluble in petroleum, cold acetone, alcohol, water, or glacial acetic acid, but readily soluble in ether, chloroform, benzene, hot acetone, alcohol or glacial acetic acid.

On analysis:

0.0824 gave 0.248 CO_2 and 0.0892 H_2O. C = 82.1; H = 12.0.

$C_{20}H_{34}O$ requires C = 82.8; H = 11.7 per cent.

When a small amount of the substance was dissolved in a little chloroform, a few drops of acetic anhydride added, and subsequently one drop of sulphuric acid introduced, a transient rose color was produced, changing successively to blue, green, and, on long standing, to brown.

When mixed with an equal quantity of the alcohol obtained from Kô-sam seeds by Power and Lees (*loc. cit.*), the melting-point was unchanged. As these two alcohols are therefore identical, on consultation with these authors it has been decided to designate it *rhamnol*, with reference to its isolation from *Rhamnus purshianus*. A determination of its specific rotation in chloroform gave the following result:

$a_D^{22^\circ C.} = -1^\circ 20'$; c = 4.296; l = 1 dcm.; $[a]_D^{22^\circ C.} = -31^\circ$.

Power and Lees found $[a]_D^{23^\circ C.} = -37.7^\circ$.

The acetyl compound was prepared by acetylation with acetic anhydride and sodium acetate, and, after recrystallization from alcohol, melted at 117° C.

On analysis:

0.2002 gave 0.5896 CO_2 and 0.2020 H_2O. C = 80.3; H = 11.2.

$C_{22}H_{36}O_2$ requires C = 80.0; H = 10.9 per cent.

Rhamnol is isomeric with, and appears to be closely related to, quebrachol, cupreol and cinchol. It differs only in melting-point from quebrachol, with which it is possibly identical, but until this point can be definitely settled it will be designated as rhamnol.

As already stated, the ethereal extract, after one crystallization from alcohol, melted at 131° C., and as the melting-point after repeated crystallization could only be raised to 135°–136° C., it was clear that only a very small quantity of an impurity could be present.

The mother-liquors from the rhamnol were concentrated and carefully

searched for any other substances, but without success. The residue from these mother-liquors was therefore converted into the acetate, as much as possible of the rhamnol acetate allowed to crystallize out, and the mother-liquors then hydrolyzed. Finally, a very small amount of an oily residue was obtained, from which on long standing a few crystals separated, which melted at 80°–90° C. indefinitely, and were evidently impure. The only neutral substance thus isolated was rhamnol.

No indication could be obtained of the existence of dodecyl alcohol, stated to have been isolated from the fat by Dohme and Engelhardt (*loc. cit.*).

Acid Constituents of the Fat.

The potassium soap, after extraction with ether, was next dissolved in water and acidified with sulphuric acid, when a precipitate was produced. The whole was then steam distilled. The acid distillate was neutralized by digestion with barium carbonate, filtered, and the aqueous solution of the barium salt evaporated to dryness. A small amount of a crystalline residue was obtained which quickly reduced silver nitrate, and when warmed with mercuric chloride in acid solution yielded calomel. The volatile acid therefore contained *formic acid.* The acid liquid remaining after the steam distillation was extracted with petroleum, and the latter removed by distillation. The residue, which was a thick, almost colorless oil, on standing partly solidified. The crystals were filtered off, drained on a porous tile and crystallized, first from alcohol, and then from glacial acetic acid until of constant melting-point. The pure acid, thus obtained in white acicular crystals, melted at 75° C.

On analysis:

0.1192 gave 0.3352 CO_2 and 0.141 H_2O. C = 76.7; H = 13.1.

Arachidic acid, $C_{20}H_{40}O_2$, requires C = 76.9; H = 12.8 per cent.

The substance was therefore *arachidic acid.*

The oily acids, from which as much arachidic acid as possible had been separated, and which decolorized bromine in the cold, were converted into the lead salts by heating with excess of lead carbonate and a little water. This product was dried and extracted with ether, the ether soluble and insoluble portions respectively treated with excess of dilute hydrochloric acid, the liberated fatty acids extracted with ether, again converted into lead salts and extracted with ether, and the acids regenerated as before.

The acids from the lead salts insoluble in ether were obtained as an amorphous mass melting at 48° C., and could not be crystallized from alcohol, as they separated in the form of a jelly.

When dissolved in chloroform and allowed to stand, crystals separated, which, after recrystallization from glacial acetic acid, melted at 74°–75° C., and were therefore arachidic acid.

The chloroform mother-liquor was then evaporated to dryness, and the residue crystallized from glacial acetic acid, when crystals were obtained melting at 64° C., but after recrystallization at 70° C. The final glacial

acetic acid mother-liquor was precipitated with water, and the precipitate collected and dried.

It melted at 51–52° C., and on analysis:

0.1072 gave 0.292 CO_2 and 0.118 H_2O. C = 74.3; H = 12.2.

Myristic acid, $C_{14}H_{28}O_2$, requires C = 73.7; H = 12.3 per cent.

Myristic acid melts at 54° C., and the melting-point and analytical numbers of the acid last obtained proves that the saturated fatty acid accompanying arachidic acid is, in all probability, myristic acid.

No evidence of the occurrence of palmitic or stearic acids, as indicated by Dohme and Engelhardt, could be obtained.

The acids from the lead salts soluble in ether formed an oily liquid which showed at first no tendency to crystallize, so they were fractionally distilled under 50 Mm. pressure. The following fractions were collected: (1) below 261° C., (2) 261°–266° C., (3) above 266° C. These fractions were then separately examined.

Fraction 1. B. p. below 261° C. under 50 Mm.

This was a light-brown oil which, on standing, deposited a few crystals, but these were insufficient to admit of further examination. The oil had a distinct smell of linolic acid, and did not give the elaidic acid reaction with nitrous acid.

On analysis:

0.0978 gave 0.2658 CO_2 and 0.1056 H_2O. C = 74.1; H = 12.0.

0.4584 absorbed 0.52 iodine by Hübl's method. Iodine number = 113.4.

Fraction 2. B. p. 261° to 266° C. under 50 Mm.

This only showed a tendency to deposit crystals when exposed to a low temperature and after standing for some time. It was similar in appearance and physical properties to Fraction 1, and on analysis:

0.092 gave 0.2584 CO_2 and 0.0944 H_2O. C = 76.6; H = 11.4.

0.3734 absorbed 0.53 iodine by Hübl's method. Iodine number = 141.9.

Linolic acid, $C_{18}H_{32}O_2$, requires C = 77.1; H = 11.4 per cent. and iodine number = 181.4.

Fraction 3. B. p. above 266° C. under 50 Mm.

This was a darker-colored oil, which deposited a few crystals on standing, and on analysis:

0.1126 gave 0.313 CO_2 and 0.1134 H_2O. C = 75.8; H = 11.2.

0.4404 absorbed 0.61 iodine by Hübl's method. Iodine number = 138.5.

These results indicate that the chief constituent of the oily acids obtained from the ether soluble lead salts was linolic acid, but that small amounts of saturated acids and possibly of the oxidation products of linolic acid were also present.

The fatty oil isolated from cascara, on hydrolysis, therefore yielded *rhamnol*, $C_{20}H_{34}O$, an alcohol of the quebrachol type, identical with

that previously isolated from Kô-sam seeds, together with *arachidic* and *linolic* acids and a small quantity of (probably) *myristic* acid. No evidence was obtained of the existence of any other acid or alcohol, or of chrysarobin and chrysophanic acid, which would have been extracted by petroleum and contained in the fatty oil examined.

2. *The Resin.*

This was a very dark brown powder which seemed to consist of two fractions, one being less soluble in alcohol than the other. These were therefore separated by extracting with a small quantity of hot alcohol, and pouring the alcoholic solution into excess of water. As, however, the subsequent chemical examination revealed no difference in behavior between the two fractions, the details of the examination of one will suffice.

The powdered resin was extracted in a Soxhlet apparatus with ether, chloroform and alcohol, successively.

The *ether extract* was a brownish syrup which deposited crystals on standing. It was dissolved in glacial acetic acid, and from this solution crystals separated which melted at 250° C., were completely soluble in dilute ammonia with a characteristic purplish-red coloration, and were therefore emodin.

The mother-liquors gave, on long standing, some more emodin, and, on adding water, a black, resinous precipitate, from which nothing definite could be isolated.

The *chloroform extract* gave a very small residue from which no definite product could be separated.

The *alcoholic extract* was a very dark brown liquid which showed no tendency to crystallize. It was poured into water, and the precipitated resin collected and dried. It was then fused with five times its weight of potassium hydroxide, the fused mass dissolved in water, acidified with sulphuric acid, and extracted several times with ether. The ethereal extract was then washed with water and distilled. The residue, which became crystalline on standing, was drained on a porous tile, and the crystals recrystallized from hot water. They then melted at 193–194° C., and their aqueous solution gave with ferric chloride a dark green coloration, which, on the addition of a drop of sodium carbonate solution, turned to blood-red. The crystals were, therefore, protocatechuic acid. The resin was thus found to contain some emodin and amorphous substances from which protocatechuic acid was obtained by fusion with potassium hydroxide.

3. *The Chloroformic Extract.*

The chloroform was distilled off, and the residue, which was partly crystalline, was crystallized from glacial acetic acid. Fine, reddish-brown, acicular crystals were thus obtained, which melted at 250° C., and, with ammonia, gave the characteristic emodin reaction. On analysis:

0.1616 gave 0.3896 CO_2 and 0.0604 H_2O. C = 65.8; H = 4.1.

Emodin, $C_{15}H_{10}O_5$, requires C = 66.6 ; H = 3.7 per cent.

From the mother-liquors, after concentration and long standing, a further quantity of emodin was obtained. When no more crystals could be obtained, a large excess of water was added to the mother-liquors, and the dark yellow precipitate formed filtered off, dried, mixed with sand, and extracted in a Soxhlet apparatus, successively, with (i) petroleum, (ii) ether, and (iii) benzene.

The *petroleum extract* was distilled and the brick-red crystalline residue recrystallized from ethyl acetate, when a very small quantity of crystals was obtained, which, after further recrystallization from benzene, melted at 200° C., and were insoluble in ammonia, but gave a faint red coloration with potassium hydroxide. The amount obtained was insufficient for analysis, but its general characters leave little doubt that it was an anthraquinone derivative allied to emodin, chrysophanic acid, etc. The mother-liquors were worked up, the resulting product freed from emodin by ammonia, and the insoluble portion acetylated. The acetyl compound, after recrystallization from 90 per cent. alcohol, melted at 155° C. Although it was not possible to identify this substance, it was quite evident that it was not chrysarobin, which melts at 200° C., gives no coloration with ammonia, a yellow solution with potassium hydroxide, and an acetyl derivative melting at 234° C.

The *ether extract* was distilled, and the residue dissolved in hot 90 per cent. alcohol. On cooling, crystals separated which melted at 180° C. These were freed from traces of emodin by dissolving in dilute ammonia and filtering; the insoluble portion, after recrystallization from several solvents, melted at 183°–184° C., and, on analysis :

0.0496 gave 0.1202 CO_2 and 0.0194 H_2O. C = 66.1 ; H = 4.3.

$C_{15}H_{10}O_5$ requires C = 66.6 ; H = 3.7 per cent.

The acetyl compound, prepared in the usual way with acetic anhydride and sodium acetate, was recrystallized from alcohol, and formed yellow, acicular crystals melting at 168° C. This substance agrees best in its properties with an isomer of emodin isolated by Thorpe and Miller from *R. frangula.**

The *benzene extract* on distillation left a residue which was too small to admit of further examination.

The examination of the chloroform extract had therefore shown that it contained emodin, an isomer of emodin, an anthraquinone derivative melting at 200° C. but not identified, and, furthermore, that no chrysarobin or chrysophanic acid could be isolated from it.

4. *The Lead Acetate Precipitate.*

The precipitate, after washing with water, was suspended in a little water, and the lead removed by hydrogen sulphide. After filtering off the lead sulphide, the aqueous liquid, which was light-brown in color, was con-

* *J. C. S.*, 1892, *61*, 6.

centrated by evaporation in a vacuum, during which process a large quantity of a black resin separated, which was filtered off. The concentrated filtrate was allowed to stand, when it slowly deposited a very small quantity of colorless crystals, which were found to consist of calcium sulphate. As no more crystals separated, but after spontaneous evaporation a hard resinous mass was obtained, this residue was dissolved in a small quantity of water and shaken several times with ether, and subsequently with benzene.

The *ethereal extract*, on evaporation, gave a very small amount of a dark-red crystalline residue, which, on further examination, was found to consist chiefly of emodin.

The *benzene extract* was so small that it was not further examined. The aqueous liquid, after extraction with benzene, was evaporated to dryness with sawdust, and the residue extracted in a Soxhlet apparatus with (1) ethyl acetate, (2) acetone, (3) alcohol, and finally (4) with water.

The *ethyl acetate extract* gave, after removal of the solvent, a small amount of a brown, syrupy residue, which showed no tendency to crystallize. A portion, dried in a vacuum over sulphuric acid, formed a sticky, very hygroscopic mass, sparingly soluble in ethyl acetate or alcohol, but readily soluble in water. Its aqueous solution gave a red coloration with ammonia, a dark-brown one with ferric chloride, reduced Fehling's solution abundantly, and remained clear on the addition of acid, but became cloudy on boiling the acid solution. No crystals could be isolated from it.

The *acetone extract* gave a very small residue, and its aqueous solution afforded reactions similar to those of the ethyl acetate extract.

The *alcoholic extract* was very dark colored, and gave no crystals. Its aqueous solution was colored deep-brown with ferric chloride, deep-red with ammonia, and only slightly reduced Fehling's solution.

The *aqueous extract* gave only very slight reactions with the above-mentioned reagents.

As nothing crystalline could be isolated from the ethyl acetate, acetone or alcoholic extracts, they were mixed in aqueous solution and allowed to evaporate spontaneously. No crystals, however, separated, and the residue was therefore dissolved in alcohol and heated with a little sulphuric acid in a reflux apparatus. The alcohol was distilled off, the residue poured into a large volume of water, and filtered.

The precipitate and filtrate will be designated A and B respectively. The precipitate, A, after drying, was obtained as a dark-brown, resinous powder, which was extracted in a Soxhlet apparatus with petroleum, ether, chloroform and alcohol successively.

The *petroleum extract* was too small to admit of further examination.

The *ethereal extract* gave, on distillation, a dark-brown residue, which was dissolved in hot glacial acetic acid. On long standing, a few crystals of emodin separated, but the amount was very small, considerably less than 1 per cent. of the product taken.

The *chloroform extract* left a dark, amorphous residue, from which nothing crystalline could be separated.

The *alcoholic extract* was similar in character to the chloroform extract.

The two latter residues were mixed and fused with potassium hydroxide, but the only definite principle that could be isolated was a very small amount of protocatechuic acid (m. p. 193°–194° C.).

The filtrate (B) was extracted several times with ether, and the ether distilled off. The residue consisted of crystals mixed with a small amount of a red oil, insoluble in water. The crystals were separated, and recrystallized twice from water, when they melted at 204°–205° C.; they proved to be identical with those obtained from the filtrate from the lead subacetate precipitate, and were therefore syringic acid (3.5 dimethylether of gallic acid).

The only definite product isolated directly from the lead acetate precipitate was, therefore, emodin.

After treatment with acid, a further trace of emodin and some syringic acid was isolated, but no other definite product. No clue was obtained as to the source of the syringic acid, and the fact that the product, before hydrolysis, contained substances which reduced Fehling's solution, rendered it impossible to say whether a glucoside was present or not.

With regard to the small amount of emodin found after treatment with acid, and in consideration of the difficulty in completely extracting it from the accompanying substances. it must be concluded that it was not formed by the acid treatment but pre-existed as such. There is, therefore, no evidence that the lead acetate precipitate contained a glucoside yielding emodin on hydrolysis.

5. *The Lead Subacetate Precipitate.*

The bulky red precipitate was well washed with water and decomposed with hydrogen sulphide in the usual manner. The filtrate from the lead sulphide was a very dark brown liquid, and was concentrated by evaporation in a vacuum. The syrupy extract obtained showed no tendency to crystallize, even after long standing. It was, therefore, thoroughly extracted, first with ether, and then with benzene.

The *ethereal extract* was distilled, and the residue gave a small amount of emodin.

The *benzene extract* was too small to admit of further examination. The residue, after extraction with ether and benzene, was mixed with sawdust, evaporated to dryness, and extracted in a Soxhlet apparatus successively with ethyl acetate, acetone, alcohol and water.

The *ethyl acetate extract* gave a dark-red residue, which, when dried in a vacuum over sulphuric acid, was found to be sparingly soluble in ethyl acetate, but readily soluble in alcohol or water. Despite numerous attempts, no crystals could be obtained.

The aqueous solution was colored dark-brown with ferric chloride, red

with ammonia, and reduced Fehling's solution. The acid solution became turbid on boiling. As will be shown later, the active constituents of the drug appear to be contained in this extract.

The *acetone extract* gave a very small residue which was similar in all respects to that obtained from the ethyl acetate extract.

The *alcoholic extract* gave a dark red residue, and constituted the greater portion of the subacetate precipitate. It showed no signs of crystallizing, even on long standing, was readily soluble in water, and behaved in most respects like the ethyl acetate residue, except that it only slightly reduced Fehling's solution.

The *aqueous extract* afforded a very dark colored residue, which gave no coloration with either ferric chloride or ammonia, and only slightly reduced Fehling's solution. It gave a dirty black precipitate with lead subacetate, and was not further examined. As no crystals could be obtained, the ethyl acetate and alcoholic residues were separately treated with alcoholic hydrochloric acid in a reflux apparatus, the alcoholic solution poured into excess of water, and the dense, greenish-black precipitate produced in each case examined as follows:

1. *Ethyl acetate extract.* This precipitate was dried and extracted in a Soxhlet apparatus with petroleum, ether, benzene and alcohol, successively, but in no case could any crystals be obtained, even after solution of the respective residues in glacial acetic acid. No trace of emodin, chrysophanic acid, chrysarobin, or other crystalline substance was found. The residues were therefore collected and mixed with those from the alcoholic extract, and the combined residues fused with potassium hydroxide.

2. *Alcoholic extract.* This was treated in exactly the same way as the ethyl acetate extract, and from the petroleum residue a small amount of a crystalline substance was obtained which gave the emodin color reaction with ammonia. The crude crystals weighed less than 0.05 gramme, and in this case must be considered to have been present in the original extract, and not to have been formed by hydrolysis. The other extracts gave no trace of crystalline substance.

Fusion with potassium hydroxide. The mixed residues (1 and 2) were fused with five times their weight of potassium hydroxide, and from the products of fusion a small amount of protocatechuic acid (m. p. 193° C.) was isolated. No other definite substance was obtained. The examination of the lead subacetate precipitate showed, therefore, that no crystalline product, or anything corresponding to Dohme and Engelhardt's purshianin, could be isolated from it, and that no evidence could be obtained of the existence of any glucoside yielding emodin or chrysophanic acid on hydrolysis.

6. *The Filtrate from the Lead Subacetate Precipitate.*

This was freed from lead by hydrogen sulphide in the usual manner,

evaporated to a low bulk in a vacuum, and set aside. No crystals separated, even on long standing, and a thick, dark-colored syrup was obtained.

A portion of the liquid, decolorized with animal charcoal, when warmed with phenylhydrazine in acetic acid solution, yielded a crystalline osazone melting at 210° C, which was therefore phenylglucosazone. The liquid thus contained a considerable quantity of glucose. It gave no coloration with ferric chloride, no precipitate with tannic acid, and when shaken with chloroform the chloroform extract gave an insignificant residue. As the liquid was very bitter, and considering that the bitter principle possibly possesses acidic characters,* it was mixed with magnesium oxide and filtered. The precipitate was well washed with water, dissolved in dilute sulphuric acid, neutralized with sodium hydroxide, and then evaporated to dryness on sawdust and extracted in a Soxhlet apparatus with alcohol. The alcoholic extract was distilled, and the residue again taken up with sawdust and extracted successively in a Soxhlet apparatus with ether, chloroform and alcohol. The ether and chloroform extracted a mere trace.

The alcoholic extract gave a dark-brown residue, which was exceedingly bitter. All attempts to obtain it in a crystalline condition were unsuccessful. It was, therefore, treated with 10 per cent. aqueous sulphuric acid in a reflux apparatus, when a dark resinous precipitate was formed. The filtrate from this precipitate was extracted with chloroform, which, after distillation, left a small amount of amorphous residue. This could not be crystallized, but its dilute aqueous solution was colored dark-brown with ferric chloride. No definite product could be isolated at any stage.

The filtrate from the magnesia, in which the bitter taste was less pronounced, gave a very slight coloration with ferric chloride, and no coloration with ammonia.

It was treated with 10 per cent. aqueous sulphuric acid in a reflux apparatus, when a tarry mass was formed. This was filtered off, and the filtrate extracted with chloroform. The chloroform, after distillation, left a brownish residue, which became crystalline on standing. It was drained on a porous tile, and recrystallized first from glacial acetic acid, and then from water, until its melting-point remained constant. It was thus obtained in white acicular crystals, melting at 206° C. (corr.,), sparingly soluble in cold water or glacial acetic acid, but much more soluble in the hot solvents. Its aqueous solution was acid to test paper, gave with ferric chloride a brown coloration, and the crystals dissolved in sodium carbonate solution with effervescence, but were reprecipitated on addition of acid.

On combustion it sublimed, forming silky needles, and

0.1241 gave 0.248 CO_2 and 0.0564 H_2O. C = 54.5; H = 5.1.

Syringic acid, $C_9H_{10}O_5$ required C = 54.5; H = 5.0 per cent.

Nitric acid produced a deep-red color, soon changing to yellow. These

* Cf. White and Robinson, Year-Book of Pharmacy, 1902, 420.

characters and the analytical figures indicate that the substance was syringic acid (3.5 dimethyl ether of gallic acid), the melting-point of which has been recorded as 202° C.

It is evident that the syringic acid does not exist as such in the bark, but in a state of combination, the nature of which cannot be indicated.

The filtrate from the lead subacetate precipitate therefore contains glu cose and a substance yielding syringic acid on hydrolysis.

EXAMINATION OF SPECIMEN 2. THE MATURED BARK.

The examination of the bark was conducted, with slight modifications, in the same manner as specimen 1. Only the results and the details of the modifications employed will, therefore, be given. The bark gave 4.9 per cent. of ash and 26.4 per cent. of dried aqueous extract.

The tannin absorbed by hide powder was found to be 3.0 per cent. The amounts yielded to various solvents were as follows:

(1) Petroleum (b. p. 40°–50° C.) = 2.4 per cent.
(2) Benzene = 1.5 per cent.
(3) Ethyl acetate = 22.3 per cent.
(4) Alcohol = 5.8 per cent.

The petroleum extract when tested for chrysophanic acid or chrysarobin gave a negative result.

The benzene extract, after crystallization from glacial acetic acid, yielded emodin equal to 0.17 per cent. of bark taken. A considerable quantity of the bark was completely extracted with petroleum, the marc dried, and then extracted with benzene. These residues were further examined.

An aqueous extract of the bark was evaporated to dryness and then extracted with alcohol. The alcoholic extract was precipitated with lead acetate and subacetate, and examined as previously described.

1. *Petroleum Extract.*

The residue, after distillation, was dissolved in hot alcohol, when, on standing, colorless crystals separated, which by re-crystallization from alcohol were resolved into a crystalline portion and a gelatinous mass. The crystals, after recrystallization from glacial acetic acid, melted at 75° C., and, on analysis, proved to consist of arachidic acid:

0.088 gave 0.2478 CO_2 and 0.1026 H_2O. C = 76.8; H = 12.9.

Arachidic acid, $C_{20}H_{40}O_2$, requires C = 76.9; H = 12.8 per cent.

The gelatinous mass, which dried to a wax, was hydrolyzed with alcoholic potash and yielded rhamnol (m. p. 135° C.) and arachidic acid (m. p. 72° C.). The portion of the fat which separated out in a crystalline condition therefore consisted of arachidic acid and rhamnol arachidate.

No evidence of the presence of chrysophanic acid or chrysarobin could be obtained.

2. *Benzene Extract.*

This left a dark-brown, partly-crystalline residue, which was recrystallized from glacial acetic acid. The dark brownish-red crystals which separated melted at 257° C., and on analysis :

0.1406 gave 0.344 CO_2 and 0.0512 H_2O. C = 66.7 ; H = 4.0.

Emodin, $C_{15}H_{10}O_5$, requires C = 66.6 ; H = 3.7 per cent.

The acetyl derivative, prepared in the usual way with acetic anhydride and sodium acetate, melted at 192° C.

As the mother liquors would contain the greater portion of the chrysarobin or allied substances present in the bark, a special search was made for them. The glacial acetic acid mother-liquor was precipitated with water and the precipitate dried. It was completely soluble in cold sodium carbonate solution, and thus was free from chrysophanic acid or chrysarobin. Other methods were tried, but no evidence could be obtained of the presence of these substances.

3. *The Alcoholic Extract.*

The lead acetate precipitate was similar in character to that previously described, it yielded emodin, and, after hydrolysis, syringic acid (m. p. 205° C.), which was analyzed :

0.0264 gave 0.052 CO_2 and 0.0124 H_2O. C = 53.7 ; H = 5.2.

Syringic acid, $C_9H_{10}O_5$, requires C = 54.5 ; H = 5.0 per cent.

The lead subacetate precipitate yielded a little emodin, but, after hydrolysis, no trace of a crystalline product.

The filtrate from the lead subacetate precipitate gave a phenylglucosazone, melting at 207°–208° C., and, after hydrolysis, some crystals of syringic acid.

The chemical examination of the fresh and mature bark of *R. purshianus*, therefore, revealed no points of difference.

EXAMINATION OF SPECIMEN 3. RHAMNUS CALIFORNICUS.

The examination of this bark was conducted in precisely the same manner as specimen 1, and therefore the results only of the examination need be given.

The bark gave 6.1 per cent. of ash and 28.2 per cent. of dried aqueous extractive.

The tannin absorbed by hide powder was found to be 3.9 per cent. The amounts yielded to various solvents were as follows :

(1) Petroleum (b. p. 40° to 50° C.) = 1.0 per cent.
(2) Benzene = 1.2 per cent.
(3) Ethyl Acetate = 19.5 per cent.
(4) Alcohol = 5.1 per cent.

The petroleum extract when tested for chrysophanic acid or chrysarobin gave a negative result.

The alcoholic extract (1300 grammes taken) yielded the following products :

1. The fat was hydrolyzed and yielded rhamnol (m. p. 135° C.) and arachidic acid (m. p. 73° C.), together with other oily acids not further examined.

2. The resin was similar in character to that obtained from *R. purshianus*.

3. The chloroform extract was crystallized from glacial acetic acid, and yielded emodin, m. p. 250° C. The amount of crystalline emodin obtained was 0.13 per cent. of the extract taken. The mother-liquors were precipitated with water, but nothing definite could be isolated.

4. The extract from the lead acetate precipitate contained a little emodin, which was extracted by chloroform; the residual extract was hydrolyzed, and a very small amount of emodin (m. p. 250° C.) obtained (.001 per cent. of the extract taken), together with some syringic acid (m. p. 204° C.). No other definite product was isolated.

5. The lead subacetate precipitate behaved exactly like that previously described under *R. purshianus* (specimen 1).

6. The filtrate from the lead subacetate precipitate yielded an osazone melting at 208°–209° C., and therefore contained glucose. After hydrolysis with sulphuric acid and extraction with chloroform, a crystalline residue was obtained, which, after re-crystallization from water, formed colorless, acicular crystals, melting at 205° C. Their aqueous solution gave a brown coloration with ferric chloride, whilst the crystals afforded with nitric acid a red coloration, changing to yellow. The substance was, undoubtedly, syringic acid. There would, therefore, appear to be no difference between the constituents of *R. purshianus* and *R. californicus*. The slight difference observed in the amount of ash, extractives, etc., afforded by the two species, might occur in barks of the same species obtained under different conditions, and no importance should be attached to them.

REPETITION OF EXPERIMENTS OF CERTAIN PREVIOUS INVESTIGATORS.

Prescott's crystals. In order to determine, if possible, the nature of the crystals described by Prescott (*loc. cit.*) the experiment was carried out exactly according to his description. One hundred grammes of the powdered bark (specimen 1) were first extracted with ether, the marc then extracted with alcohol, the alcoholic solution poured into water, filtered, and the filtrate precipitated with lead acetate solution. This precipitate was then suspended in absolute alcohol and decomposed by hydrogen sulphide, the lead sulphide filtered off, and the very dark-colored alcoholic filtrate allowed to evaporate spontaneously. The solution first deposited an amorphous, brown substance, which was separated, and on further concentration a brown, amorphous varnish was obtained. No crystals could be observed or isolated.

Le Prince's cascarine. Attention has already been directed (p. 3) to the probability of this substance consisting of impure emodin. The details of its preparation, as given by Le Prince in his original paper (*loc. cit.*),

have therefore been carefully carried out on two separate quantities of material by the author, and also, independently, by another worker in the laboratories with the following result:

500 grams of the powdered bark (specimen 1) were extracted with a hot 10 per cent. aqueous solution of sodium carbonate, the mass strained, and the liquid neutralized with sulphuric acid. The neutral liquid was then evaporated to dryness in a vacuum and extracted with acetone. The acetone solution, which was somewhat dark-colored, was then concentrated by distillation, and the extract acidified with a little sulphuric acid and allowed to stand for six hours. It was then poured into a large volume of water and allowed to stand for 24 hours. A brownish-yellow, amorphous precipitate almost immediately separated, but even after standing it showed absolutely no trace of crystalline structure. It was filtered off and dried, and was then found to melt very indefinitely at about 190° C., not at 300° C. as stated by Le Prince. The yield of dried product was 1.1 grams or 0.22 per cent., and it was very similar in appearance and general properties to the resin previously described. Attempts to crystallize it from glacial acetic acid being unsuccessful, it was extracted with hot benzene, and the benzene solution distilled. The residue was dissolved in hot glacial acetic acid, and on standing it deposited a few crystals of emodin, identified by the melting-point and the color reaction with alkalies. The cascarine of Le Prince consisted, therefore, of resin with a very small amount of emodin, and the formation of protocatechuic acid by fusion of the resin with potassium hydroxide has already been noted (p. 14).

Le Prince's isolation of chrysophanic acid and chrysarobin. Although, as has already been stated, it was found impossible to obtain any indications of the existence of chrysarobin or chrysophanic acid in the bark, it was deemed of importance to repeat Le Prince's experiment in this connection.

500 grammes of the powdered bark were macerated with a five per cent. aqueous solution of sodium hydroxide, and the strained liquid precipitated with sulphuric acid. The precipitate was then dried and extracted in a Soxhlet apparatus with acetone, the acetone solution poured into water, and the brownish-black precipitate collected and dried. This product was again subjected to extraction with acetone and subsequent precipitation with water. The amorphous, black residue was first extracted with a very small quantity of cold glacial acetic acid and filtered. The filtrate, which, according to Le Prince, should contain chrysarobin, deposited no crystals on standing. It was therefore poured into water, and the precipitate collected and dried. It was then digested with hot benzene, when only a small portion dissolved, and the benzene solution deposited no crystals on cooling and standing. A further extraction of the residue with hot ethyl acetate and subsequent filtration gave no crystalline product.

The residue, insoluble in a small quantity of cold glacial acetic acid, was

treated with a large quantity of this solvent and filtered. The filtrate, which would contain any chrysophanic acid present in the drug, deposited no crystals on standing. It was poured into a larger quantity of water, the precipitate collected, dried and dissolved in hot 90 per cent. alcohol. On cooling the solution and allowing it to stand for several days, no trace of crystals was obtained.

The repetition of Le Prince's experiments, therefore, served only to confirm the results arrived at by the other methods, and proved that no chrysophanic acid or chrysarobin could be isolated from the bark.

PHYSIOLOGICAL EXPERIMENTS WITH PREPARATIONS OF THE BARK.

Experiments were first made to determine whether emodin is the active principle of the drug, or if it exerts any purgative action. For this purpose pure emodin was administered to several persons, but even in one grain doses, it was found to be quite inactive. As it was possible that, although inactive under the above conditions, it might be active when associated with the other constituents of the bark, an aqueous solution of the alcoholic extract was divided into two portions, and one of these extracted by chloroform, by which means the emodin was removed. The two solutions were then administered in equal doses, and as far as possible, under similar conditions, but no difference in their action could be detected. The emodin present in cascara, therefore, would appear to exert no purgative action. In order to determine whether the active principle could even approximately be located, the following preparations were physiologically examined. As by the previously described method of procedure, it was probable that only partial separation had been effected, it was to be expected that no sharp distinction in their action would be observed, but that the presence of the active principle would be revealed by the greater activity of one of the preparations. The preparations examined and the results obtained were as follows:

Regenerated lead acetate precipitate—very slightly active.

Regenerated lead subacetate precipitate.

(1) Portion extracted by ethyl acetate—very active.

(2) Residue extracted by alcohol—slightly active.

Filtrate from lead subacetate precipitate—inactive.

The above were given in 7 Cc. (2 drachm) doses of a 10 per cent. aqueous solution.

These results indicate, somewhat conclusively, that the active principle as principles of cascara are contained in that portion of the alcoholic extract which is soluble in water and precipitated by lead subacetate.

Furthermore, it is contained in that portion of the regenerated lead subacetate precipitate which is soluble in ethyl acetate.

The hydrolytic enzyme from the bark was given in 1 gramme doses to a dog and to a man. It was inactive—except for a very slight aperient effect in the man—and there was no indication whatever of griping or nausea at any stage.

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